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A TRYPANOSOME OF PANAMANIAN CATTLE AND A METHOD FOR CONCENTRATING TRYPANOSOMES IN PERIPHERAL BLOOD

OSCAR TEAGUE AND HERBERT C. CLARK

From the Board of Health Laboratory, Ancon, Canal Zone

We have been able to show that a large percentage of the beef cattle brought to Panama City from various parts of the Republic of Panama carry in their blood a large trypanosome. Since these animals are apparently in good health, the presumption is that the trypanosome is nonpathogenic; however, another possibility is that the trypanosome is pathogenic, but that the cattle have developed an immunity against it.

The trypanosome could not be found in direct smears of the blood, but developed in cultures grown at 26 C. For this purpose 2 c.c. of the freshly drawn and defibrinated blood were added to 10 c.c. of nutrient broth containing 0.85% of sodium chlorid. The trypanosome can usually be detected in the upper layer of the sediment (the leukocytic layer) of the culture after 24-48 hours' incubation. On the 3rd or 4th day they are generally present in large numbers, occurring in clusters with the flagella extending outward and the posterior ends embedded in a central clump of leukocytes. The trypanosomes remain alive and actively motile in these cultures for 30 days and longer. After a few days, however, marked changes in morphology occur; along with forms resembling trypanosomes, but varying markedly in size, are others like crithidia, some oval flagellated bodies, and even spermatozoan-like forms.

Obviously the cultures were unsatisfactory for studying the morphology of the trypanosome. Since smears from the blood of cattle known to be positive by the culture method were negative and laboratory animals inoculated with the cultures of the trypanosome and with cattle blood did not show trypanosomes in their blood, there appeared to be only one possible way of accurately determining the morphology of the trypanosome, namely, by destroying the red cells and then concentrating the trypanosomes in laked blood by centrifugation. Several different methods were tried to accomplish this purpose, the following one being finally chosen.

The freshly drawn blood is whipped with wooden sticks in an enamel-ware cup and the defibrinated blood is poured through wire gauze to remove bits of fibrin. Ten c.c. of the blood are added to 10 c.c. of distilled water, and the two are thoroughly mixed. This was done at the slaughter house. Hemolysis of the red cells takes place in a few minutes. As soon as we reached the laboratory, the blood was centrifugated, the supernatant fluid was poured off and the sedi-

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ment was emulsified in the 2 or 3 drops of fluid remaining. Smears from this sediment showed well preserved trypanosomes in almost every instance, from 1-10 or 12 or more to a slide having been found. A drop of the sediment examined under a cover-slip occasionally showed a trypanosome, which remained actively motile for more than an hour after the distilled water had been added to the blood. There is a distinct advantage in defibrinating the blood before laking the red cells, as a smaller amount of distilled water is required and the sediment after centrifugation seems to be smaller in amount.

After we had developed this concentration method, a calf that had been inoculated 7 days previously with a large dose of cattle blood showed trypanosomes in its peripheral blood in such numbers that they were very readily found in direct smears. This furnished us with additional material for the study of the morphology of the trypanosome.

In size and morphology the trypanosome agrees with the description of Tr. theileri. In the films from the calf's blood the largest trypanosome measured by us was 4.5μ wide by 54μ long exclusive of the flagellum which was 13.5μ long; the smallest was 20.4μ long and 3.6μ wide with a flagellum measuring 16.2μ. They were usually about 45μ long by 3.5μ wide with a flagellum about 14μ. The posterior end is long drawn out and pointed. The undulating membrane is well developed. The blepharoplast is round and is generally situated one fourth the length of the body of the trypanosome from the posterior end. Some of the very small trypanosomes have the blepharoplast disproportionately near the posterior end and the flagellum is relatively longer than in the larger individuals. The blepharoplast has most often a marginal location. The nucleus stains well and lies nearly equidistant from the two ends of the trypanosome.

The trypanosomes from adult cattle obtained by the concentration method previously described were larger than those in the blood of the experimental calf and showed marked differences in their staining characteristics. That these differences were not the result of the treatment with distilled water was shown by the fact the trypanosomes in the calf's blood, when so treated, stained in the same manner as those in direct smears from the blood. The cattle trypanosomes measured from 42μ to 80μ long without the flagellum and from 3.6μ to 5.4μ wide. The nucleus is in most instances represented by a rectangular pale space occupying the entire width of the trypanosome and situated approximately equidistant from the 2 ends. In rare cases a faintly staining oval was found and in still other instances the nucleus was not visible. The trypanosome stained usually a deep blue color with the Hastings' stain. The blepharoplast occupied the same relative position as in the trypanosome of the calf. The undulating membrane was well developed, but the free flagellum was short, measuring from 7.2μ to 13.5μ; occasionally no flagellum was seen.

In spite of the differences in size and staining properties, the trypanosomes in the 2 instances are identical, the chronic infection in the cattle with slow multiplication of the trypanosome yielding large individuals, while the acute infection in the calf with rapid multiplication gave rise to smaller organisms. These observations suggest that possibly Tr. ingens is identical with Tr. theileri, since the staining characteristics of this organism closely resemble those of the trypanosome in our cattle; however, the maximum measurements of Tr. ingens are decidedly greater than ours.

The following animals were inoculated with young cultures of the trypanosome: a guinea-pig, a monkey, a pigeon, and a chicken. Repeated examinations of the blood of these animals during the succeeding weeks were negative for trypanosomes except in the case of the pigeon. A large trypanosome was seen in a cover-slip preparation of the fresh blood of this bird on the 11th day after inoculation, but none were found in stained films. The concentration method could not be applied to this blood, because the red cells of the pigeon are nucleated and the nuclei are not disssolved by distilled water. Cultures from the pigeon's blood failed to show trypanosomes; hence it seems probable that the trypanosome seen in the cover-slip preparation was Tr. columbae and not the cattle trypanosome with which the bird had been inoculated.

In order to eliminate the possibility of a loss of virulence of the trypanosome in the culture, the blood of cattle known to harbor the trypanosome was injected subcutaneously into the following animals: 4 guinea-pigs, 1 chicken, 2 pigeons, 1 monkey, 3 opossums, 2 puppies, 1 goat and 1 mule. One trypanosome was seen in a cover-slip preparation of blood of 1 of the pigeons on the 5th day after inoculation, but this was in all probability Tr. columbae. The results were entirely negative in the other instances; the animals showed no signs of illness and trypanosomes were not demonstrated in their blood. For each group of the animals mentioned that were inoculated at the same time, the mixed, defibrinated blood of 2 or 3 steers was used.

In contrast to these results the inoculation of a young calf yielded particularly interesting results and the protocol of this experiment will be given in detail.

A heifer calf about 4 months old from Corozal Farm was inoculated with smallpox vaccine on June 27, 1917, and the vaccine was collected on July 3, 1917.

July 8, 1917.—The calf was bled from the jugular vein and cultures were made in broth. No trypanosomes ever appeared in these cultures.

July 9.—Ten c.c. of the mixed defibrinated blood of 2 steers was inoculated into the calf subcutaneously. Trypanosomes were demonstrated in the blood of the 2 steers. Smears from the calf's blood remained negative.

July 25.—The calf was bled from the jugular vein and the blood was cultured. The cultures remained negative for trypanosomes. Smears from the calf's blood taken at intervals were negative.

Aug. 4.—The calf was given 32 c.c. of the mixed, defibrinated blood of 3 steers known to harbor the trypanosome.

Aug. 16.—The blood of the calf contains numerous trypanosomes. Bled from the jugular vein. Cultures in broth at 24-26 C. were positive and showed the same marked changes in the morphology of the trypanosome that were previously encountered in cultures from steers. The calf's blood inoculated into a puppy, a monkey, a goat and a mule yielded only negative results. The defibrinated blood treated with an equal volume of distilled water and centrifugated strikingly confirmed the value of the concentration method we had been using for the steer's blood. In stained smears of the calf's blood a trypanosome was encountered only after passing over a dozen or more fields with the oil-immersion lens; after concentration there were from 1-10 trypanosomes to a field.

Aug. 20.—Blood smears positive for trypanosomes.

Aug. 24.—Blood smears positive for trypanosomes.

Aug. 27.—Blood smears negative for trypanosomes.

Aug. 31.—Blood smears negative for trypanosomes.

The trypanosome of Panamanian cattle agrees with the description of Tr. theileri (1) in size, (2) in morphology, (3) in being readily cultivated in broth at 26 C., and (4) in being unable to develop in other animals than cattle. We have therefore concluded that it is identical with this trypanosome.

Tr. theileri was discovered in the Transvaal in 1903. It was soon afterwards found in other parts of Africa, in India and Eastern Asia. It was first cultivated by Miyajima in Japan and later by Martini in the Philippine Islands, but these authors did not recognize the trypanosomes which they studied as Tr. theileri. Martini thought he had to do with a new species partly because the distal end of the flagellum of the trypanosome which he cultivated was clubbed. This feature is quite common in our cultures, but does not occur in the trypanosomes stained directly in the blood. It is obviously dangerous to attempt to describe trypanosomes from cultures alone. Crawley obtained cultures of a large trypanosome (Tr. americanum) in the United States of America. Tr. theileri has been obtained in cultures in Germany, England, France, Denmark, Sweden, Greece, Algiers, Tunis and Uruguay. It is regarded by almost all writers as being nonpathogenic.

SUMMARY

Tr. theileri occurs in a large percentage of beef cattle in Panama.

It is present in such small numbers in the peripheral blood, that stained smears are uniformly negative.

After defibrinating the blood, treating with an equal volume of distilled water and centrifugating, we find it almost always in smears from the sediment.

Filaria are also readily demonstrated in the blood of Panamanian cattle by the same procedure, although they are very rarely found in ordinary blood films.

The trypanosome is readily cultivated in broth at 24-26 C., but undergoes marked changes in morphology in the culture. Inoculation into other species of animals than cattle yielded negative results.